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Perspective

E Pluribus Tres: The 2009 Nobel Prize in Chemistry

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This year's Nobel Prize in Chemistry celebrates a multitude of research areas, making the difficult selection of those most responsible for providing atomic details of the nanomachine that makes proteins according to genetic instructions. The Ribosome and RNA polymerase (recognized in 2006) structures highlight a puzzling asymmetry at the origins of biology.

I initially wrote this piece in response to a college classmate who, knowing something about my profession, asked if I knew any of the winners and what he should make of the 2009 Nobel Prize in Chemistry. At some levels, I know this story like the back of my hand. Much of science is being in the right place at the right time, and it is only natural that I spend a reasonable amount of time reflecting on work that I truly admire and wish that I could have participated in.

The macromolecular crystallography community has always been small, supportive, and tightly knit. The American Crystallographic Association (ACA) has around 1800 members and is the smallest but one of all of the member societies of the American Institute of Physics (AIP). As a consequence, many ACA members, me included, have enjoyed the privilege of friendship with all three of the 2009 Prize winners.

When an NPR reporter on Morning Edition woke me on October 7, 2009, with news that the winners would be revealed at 6:30 a.m. and that they were selected for "decoding DNA," I was totally stumped as to who this year's winners might be. I thought of several possibilities—people who work in my own area, but never thought about the ribosome work—and, when the announcement that the prize was awarded to Venkatraman Ramakrishnan, Thomas A. Steitz, and Ada E. Yonath was made moments later, I was genuinely surprised. I had long ago decided that this particular choice would be so difficult—so many people had made absolutely essential contributions that they couldn't select just three. I immediately sent an email to Peter Moore, whose name I thought might belong on the list, under the heading "Solomon's Decision." That short message expressed condolences and congratulations, saying I felt injured that he was not among the three.

Peter Moore, Professor of Chemistry at Yale University, is certainly an important reason that Tom Steitz (Yale University)-was able to walk through the structure of the "50S subunit" (more about that in a moment). Peter delivered a stunningly good talk at my father's 65th reunion at Yale in the summer of 2008. Dad had asked me for advice on possible speakers, and I immediately suggested Peter. Educated at both Yale and Harvard University, Peter is a spectacular scientist and a generous human being. He treated my father as if they were old friends. I went to the reunion to introduce him before his talk and deliver the Class of '43 reunion gift, a small Yale-blue Swiss Army knife.

Within minutes of receiving my email the day the Nobel prizes were announced, Peter responded, saying that he was not at all bothered. If he had been on the committee, he said, he would have made exactly the same choice. "They got it right!" he concluded. At the Yale press conference, Tom was given a generous round of applause. But when he tipped his hat to Peter Moore, the crowd erupted for a prolonged applause that went on so long that a reporter phoning in to the press conference from the AIP told me he thought his call had been dropped and he was hearing static noise. The occasional human voice made him realize that it was applause and it seemed to go on for several minutes.

Peter Moore was not the only scientist I felt had been slighted. Several others were arguably equally as deserving. Among them, the most obvious is Harry Noller (University of California, Santa Cruz), whose biochemical studies established quite early that the 23S RNA retained catalytic peptidyl transferase activity without any protein subunits (Noller et al., 1992). Demonstration that the ribosome is a ribozyme was perhaps the most profound surprise arising from the ribosome crystal structures. Others from Noller's group, principally Jamie Cate (University of California, Berkeley), also provided the first structure containing both 30S and 50S subunits in an intact 70S ribosome (Schuwirth et al., 2005; Vila-Sanjurjo et al., 2006; Yusupov et al., 2001). I also have been impressed that Richard Brimacombe and others correctly worked out the RNA tertiary structures using distance constraints from covarying bases, as if they were nuclear overhauser effects for an NMR structure determination (Mueller and Brimacombe, 1997).

So, what is the ribosome? Why were the winners so hard to select? And why would Peter Moore tell me straightaway that the Nobel Committee had "got it right"?

There are two nanomachines in the cell that, for me, are more important than any others. One makes proteins, the other makes RNA. The ribosome is the one that reads and makes proteins according to the genetic instructions. Peter Moore's brilliant metaphor is the Jacquard Loom. You want a particular pattern in the weave, so you insert a template with those instructions. The ribosome can read the instructions for making any of the cell's proteins from the corresponding template, which is a long string of RNA called "messenger RNA" or "the message." Probably the most important details of how it does this have been worked out in much detail using elegant pre-steady state kinetic analysis by people like Marina Rodnina and Wolfgang Wintermeyer (Pape et al., 1999; Rodnina and Wintermeyer, 2001). However, there is really nothing like a first-hand witness, and that requires a structure.

To "solve" an X-ray crystal structure, you need to take a series (many hundreds) of "group" photographs of crystals containing roughly 10^{15} molecules, all in rows, facing and smiling exactly the same way. The ribosome is constructed from two huge pieces, which come together to read a message and then dissociate once the job is finished. The two pieces have common names derived from their sedimentation behavior in an ultracentrifuge. Theodor Svedberg (Nobel Laurate in 1926) developed the ultracentrifuge, so the units of size are Svedberg units or S for short. The large ribosomal subunit is called the 50S; the small one the 30S. The two subunits do radically different jobs. The 30S subunit is the "brains" of the machine and is also called the "decoding" subunit because it binds to the message and directs the assembly of the parts necessary to stitch on the next amino acid in the growing polyamino acid that is to become a protein. The 50S subunit is the "brawn," a more or less inert template that provides the catalytic surface for stitching the successive amino acids together after the 30S subunit has brought the right components all into place.

With this background, it is perhaps easier to understand how each of the three Prize winners fit into the picture. Ada Yonath (Weizmann Institute of Science), whom I met for the first time in 1981 on my only visit to Israel, was at that time a very rambunctious and flamboyant figure. She had decided to purify and crystallize "the ribosome." Some were amused by her efforts because they assumed that she could never get the ribosome pure enough to crystallize (i.e., to assemble the group for the many necessary photographs). Two years later, she produced the first real crystals. The giggling continued— "These crystals will never 'diffract' because they cannot possibly be well-enough ordered." Ada proceeded to team up with Håkon Hope, a Norwegian who was interested in how cryogenic temperatures affected diffraction (Hope, 1990). This teamwork was immensely transformative, and soon everyone in the world was learning how to plunge crystals into liquid nitrogen to keep them alive in the X-ray beam. Sure enough, the 50S ribosome crystals did diffract, once they were cryoprotected. Folks still giggled: "She'll never be able to make heavy-atom derivatives."

An essential component of X-ray crystallography, and one that attracts many crystallographers to the field, is that when you collect data, you measure only half of the necessary information. For each data point, there are two components necessary to reconstruct images. They are called the A part and the B part, or the amplitude and the phase. It turns out you can only record the intensity, which is related to the amplitude, $\sqrt{(A^2 + B^2)}$. The phase, which is $\tan^{-1}(B/A)$, gets consumed in the process of recording the amplitude, and is lost. To recover the phases, you need to triangulate. And triangulation means preparing suitably derivatized crystals that have bound heavy atoms with a sufficient number of electrons to shift the amplitudes by a measurable amount related to where on the object they bind.

By this time (early 1990s), Ada routinely took small teams to each of a half of one dozen or so synchrotrons around the world, while she maintained a group of ~40 split between her home institution, the Weizmann Institute in Rehovot, and the DESY synchrotron in Hamburg. She teamed up with different organometallic chemists to synthesize clusters of gold and tungsten atoms (12-18 atoms). This eventually worked, and Ada began to get these heavy atoms to bind (Yonath, 1992).

By 1995, people began to pay attention. Among those who had taken Ada seriously from her first successes were Peter Moore and Tom Steitz, who decided that she had had the field to herself far too long and it was time to actually solve the problem. The next part of the story involves two technical developments that helped Steitz to complete the structure of the 50S subunit in an astonishingly short time. Not surprisingly, while waiting for their photographs to be taken, the 10^{15} molecules in a crystal fidget and sometimes they play a subtle game of musical chairs, which changes where you find the data points. An answer to this problem came from Moore's lab, where Betty Freeborn's studies showed that careful monitoring and control of ionic strength ensured that the 50S subunit crystals reproducibly belonged to the same space group. It turns out that Ada's critics had been right in one respect: her crystals continued to be irreproducible after all (Yonath et al., 1998).

The second technical breakthrough came from cryo-electron microscopy. Joachim Franck at the Wadsworth Institute in Albany (now at Columbia University) and Marin van Heel (Imperial College, London), had described correspondence analysis techniques that solved the problem of aligning multiple images so that they could be averaged for tomography (van Heel and Frank, 1981). Frank and van Heel began to compete intensely when both turned their attention to the ribosome. Each separately produced almost identical images of ribosomal components (Frank et al., 1995; Stark et al., 1995). Phases calculated by orienting these images relative to the diffraction patterns could be used together with the X-ray amplitudes to confirm the location of the heavy atom clusters in the crystals derived from analysis of difference Patterson maps (Ban et al., 1998). Solution of the phase problem initiated a convergent cycle of refinement that rapidly produced the 50S structure at 2.4 Å resolution, published in 2000 (Ban et al., 2000). That work was done in Steitz's lab by a brilliant post-doc, Nenad Ban, now at the ETH in Zürich.

The millennium brought dramatic successes from all three groups (Ban et al., 2000; Carter et al., 2000; Nissen et al., 2000; Schlunzen et al., 2000, 2001; Wimberly et al., 2000). The new, high-resolution structures unearthed remarkable new molecular details. And these details matter.

The 50S subunit produced two really stunning facts. First, consistent with Noller's results, there was no protein near the site where proteins are made—the machine is made almost entirely of RNA (Nissen et al., 2000). Second, RNA tertiary structure makes frequent use of the unique ability of adenine to form specific interactions in the minor groove of another double helical fragment to stabilize tertiary structures throughout, and more specifically in both peptidyl-transferase interactions on the 50S subunit and decoding interactions on the 30S subunit (Nissen et al., 2001). In addition, Ban's structure revealed details of an exit tunnel, identified at lower resolution in earlier work (Frank et al., 1995; Yonath et al., 1987), where the growing protein chain leaves the ribosome and goes on to fold and do its own thing (Ban et al., 2000). Many of the antibiotics known to act on the bacterial ribosome get stuck at the entry to that tunnel—in Peter Moore's words, "constipating it." The 50S structure explained mechanisms of action for these and other antibiotics. Bacterial ribosomes are very different from the 80S ribosomes of higher organisms, whose 50S subunit is really

60S and whose 30S subunit is 40S. That is why the bacterial ribosome is such an important target for antibacterial drugs.

Venki Ramakrishnan (now at the MRC Laboratory of Molecular Biology, Cambridge, UK) had worked with Peter Moore for some time early in his career while Moore was trying to solve the ribosome structure by another cruder method. Quite suddenly, he began producing structures of the 30S subunit. The 30S subunit is far more interesting than the 50S subunit, and Venki's work is truly spectacular, in a league by itself. As a physicist, Venki understood the problems associated with "decoding" more deeply than his contemporaries and the structures he produced revealed several more stunning new facts. (1) The 30S subunit is dynamic—its parts move relative to one another during a cycle of adding a new amino acid. (2) This movement closes a mold around the base pairs between the message and the anticodon of the tRNA that brings the next amino acid into position (Ogle et al., 2002). This mold is a rather rigid stereochemical probe, analogous to the press used by a notary public. If it closes successfully (all of the way), the codon-anticodon pair is perfect and the correct amino acid is in position. If it fails to close, the tRNA contains the wrong amino acid and is rejected. (3) A different class of antibiotics acts by promoting the closure of the mold, even for near-cognate but incorrect amino acids. All of this work is accessible at Venki's website (http://www.mrc-lmb.cam.ac.uk/ribo/homepage/ramak/index.html). I urge readers to visit these pages and look at the movies there, especially the one that shows what happens upon tRNA binding and the induced-fit mechanism of codon-anticodon recognition (http://www.mrc-lmb.cam.ac.uk/ribo/homepage/movies/trna_induced_fit.avi).

This is enough of the story to see that the Nobel Committee did not choose an Alexandrian solution to the Gordian knot, and why Peter Moore would tell me that they got it right. I've always admired Tom Steitz's work because he does not take shortcuts and gets things right. His group actually pushed the project over the goal line. The project itself would never have been born, but for Ada Yonath's vision and eclectic tenacity and her improvisations profoundly transformed all macromolecular crystallography. Venki Ramakrishnan is a wizard, and from his work we derive the deepest sense of how translational fidelity is achieved (Ramakrishnan, 2002).

All three groups have elucidated the interactions of ribosome-directed antibiotics (Franceschi and Duffy, 2006), and their contributions point toward a bright future for antibacterial drug development. A direct outgrowth of the work at Yale is the Biotech startup Rib-X, whose mission is to develop new generations of drugs to combat multidrug resistance. In this context, we can anticipate that further insights from structural differences between bacterial ribosomes and the larger, more complex, and as yet unsolved eukaryotic ribosome crystal structures will help us outrun microbial evolution, at least in the intermediate term.

It is interesting to compare the drama behind this year's awards with a similar prize awarded in 2006. The X-ray structure of RNA polymerase, the other of the cell's big nanomachines along life's information highway that makes RNA from DNA, was solved entirely by one group (Cramer et al., 2001; Gnatt et al., 2001; Wang et al., 2006)—that of Roger Kornberg (Stanford University), son of Arthur Kornberg. Both Kornbergs won Nobel Prizes; Roger's was awarded in Chemistry three years ago. In retrospect, the problems of the ribosome and RNA polymerase are somewhat similar, but the scientific histories are quite different. Roger's Nobel Prize was also controversial. However, one must, I think, acknowledge that his group legitimately combined the work done separately by the many groups who contributed to the ribosome structures. His interview, available online, eloquently describes many of the challenges faced by scientists (http://nobelprize.org/mediaplayer/index.php?id=78).

There are deeper lessons yet to be mined from the atomic coordinates left to us by this year's Chemistry Nobel laureates. There is no protein in the 50S subunit's active site, and the 30S subunit's decoding site operates largely without proteins. Jeremy Berg highlighted this observation in his email to scientists funded by NIGMS as evidence for what is called the RNA world hypothesis—that RNA preceded proteins because it was capable of doing what proteins can do, whereas proteins cannot encode heritable information. Ironically, identical logic works in reverse: the RNA polymerase structures contain no shred of RNA!! Perhaps a more profound partnership between these two biopolymers extends back much further than the RNA world hypothesis suggests (Carter and Kraut, 1974).

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